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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/12, C07K 15/06
C12Q 1/68

A2

(11) International Publicati n Number: WO 94/00572

(43) International Publication Date: 6 January 1994 (06.01.94)

(21) International Application Number: PCT/US93/05794

(22) International Filing Date: 18 June 1993 (18.06.93)

(30) Priority data:
07/903,466 22 June 1992 (22.06.92) US

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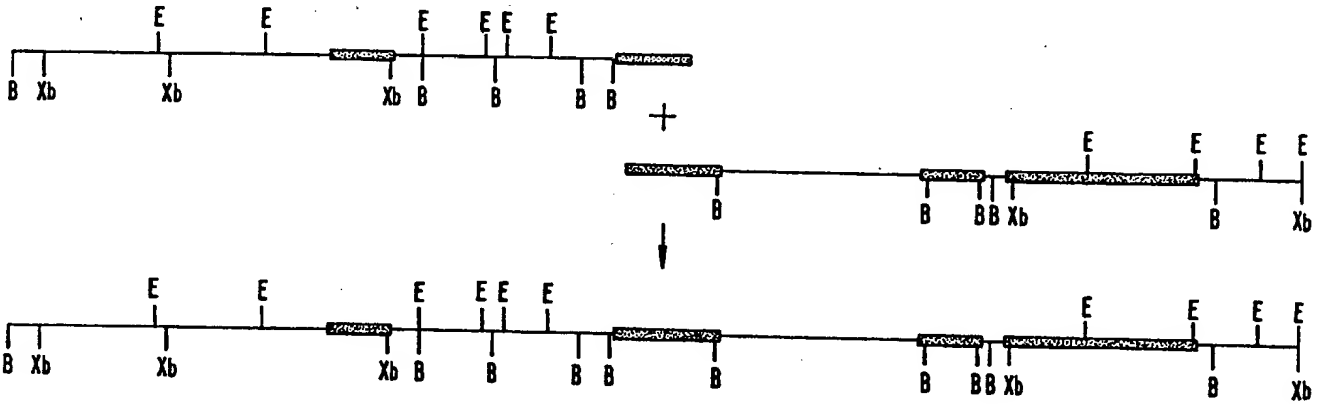
(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: GENE FOR ATAXIA-TELANGIECTASIA COMPLEMENTATION GROUP D (ATDC)



(57) Abstract

Disclosed herein is an AT gene for complementation group D, the *ATDC* gene and fragments thereof. Nucleic acid probes for said gene are provided as well as proteins encoded by said gene, cDNA therefrom, preferably a 3 kilobase (kb) cDNA, and recombinant nucleic acid molecules for expression of said proteins. Further disclosed are methods to detect mutations in said gene, preferably methods employing the polymerase chain reaction (PCR).

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GENE FOR ATAXIA-TELANGIECTASIA COMPLEMENTATION GROUP D (ATDC)

FIELD OF THE INVENTION

The present invention is in the general area of medical genetics. More specifically, it relates to the identification of a new gene--the ataxia-telangiectasia group D gene--the ATDC gene.

This invention was made with Government support under Contract Nos. DE-AC03-76-SF01012 and W-7405-ENG-48, awarded by the Department of Energy. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Ataxia-telangiectasia (AT) is a human autosomal recessive disease that exhibits progressive neuromuscular problems, immunodeficiencies, a high incidence of lymphoreticular cancer, and sensitivity to ionizing radiation [Taylor, "Cytogenetics of ataxia telangiectasia," IN: Bridges and Harnden (eds.) Ataxia-telangiectasia--a cellular and molecular link between cancer, neuropathology and immune deficiency, pp. 53-82 (Wiley, Chichester 1988); Boder, "Ataxia telangiectasia an overview," IN: Gatti and Swift (eds.), Ataxia telangiectasia: genetics, neuropathology and immunology of a degenerative disease of childhood, pp. 1-63 (Alan R. Liss; New York, 1985); and Morrell et al., J. Natl. Cancer Inst., 77: 89-92 (1986)]. AT heterozygotes, which constitute as much as 3% of the human population, have been reported to have an increased risk of cancer after exposure to ionizing radiation.

Cells from patients with AT display two hallmark characteristics: hypersensitivity to the killing effects of ionizing radiation [Taylor et al., Nature, 258: 427-429 (1975)] and resistance to the inhibiting effects of ionizing radiation on the rate of DNA synthesis, that is,

radioresistant DNA synthesis [Young and Painter, Hum. Genet., 82: 113-117 (1989)]. Thus, the identification of genes that are responsible for those abnormalities would greatly further the understanding of human radiosensitivity and the regulation of DNA replication after radiation-induced DNA damage. The characteristic of AT cells to exhibit radioresistant DNA synthesis has been used to establish the presence of several complementation groups within this disease [Jaspers and Bootsma, PNAS (USA), 79: 2641-2644 (1982); Murnane and Painter, PNAS (USA), 79: 1960-1963 (1982); Jaspers et al., Cytogenet. Cell Genet., 49: 259-263 (1988)].

Despite extensive investigation, the underlying defects responsible for the pleiotropic abnormalities presented by AT remain unknown. Genetic linkage analysis [Gatti et al., Nature, 336: 577-580 (1988); McConville et al., Nucl. Acids Res., 18: 4335-4343 (1990a); McConville et al., Hum. Genet., 85: 215-220 (1990b); Sanal et al., Am. J. Hum. Genet., 47: 860-866 (1990); and Ziv et al., Genomics, 9: 373-375 (1991)] and chromosome transfer studies [Lambert et al., PNAS (USA), 88: 5907-5911 (1991)] have shown that the gene(s) associated with three complementation groups are all located at the chromosomal region 11q22-q23.

Complementation groups A (AT-A) and C (AT-C) have been mapped by genetic linkage analysis [Gatti et al. 1988; McConville et al. 1990a and 1990b; Sanal et al. 1990; Ziv et al. 1991; and Foroud et al., Am. J. Hum. Genet., 49: 1263-1279 (1991)]. Using families from mixed complementation groups, two groups of investigators independently reported linkage of the AT gene(s) to two separate regions, one of which is near THY1 [McConville et al. 1990a and 1990b; Sanal et al. 1990; Foroud et al. 1991]. It was subsequently determined that the genes for AT-A and AT-C are located within the more centromeric of these two regions [McConville et al. 1990a; Sanal et al. 1990; Foroud et al. 1991], although it was concluded that the AT gene in a small subset of families could map to the second locus near THY1 [Sanal et al. 1990]. A study by Gatti et al. [in paper entitled "Ataxia-telangiectasia: linking evidence for genetic heterogeneity,"

presented at AACR (American Association for Cancer Research) Special Conference "Cellular Responses to Environmental DNA Damage" in Banff, Alberta (Canada) D cember 1-6, 1991] which excluded families in complementation groups A and C, concluded that a gene for an additional complementation group does show linkage to the region near THY1. The gene for complementation group D (AT-D) was a likely candidate for linkage near THY1 as complementation group D is the next most common complementation group for AT after A and C [Jaspers et al. 1988].

Functional complementation has been used to prove the identity of several genes that provide resistance to various DNA-damaging agents [van Duin et al., Cell, 44: 913-923 (1986); Thompson et al., Mol. Cell Biol., 10: 6160-6171 (1990); and Weeda et al., Mol. Cell Biol., 10: 2570-2581 (1990)].

Using the sensitivity of AT cells to ionizing radiation, Kapp and Painter [Int. J. Radiat. Biol., 56: 667-675 (1989)] attempted to complement the defect in an AT cell line (AT5BIVA) from complementation group D (AT-D) by transfection with a human cosmid library containing a selectable neo gene. The combined selection by ionizing radiation and G418 resulted in the isolation of an AT cell line (1B3) that is partially resistant (approximately 50% of normal) to ionizing radiation and produces fewer radiation-induced chromosome aberrations, but retains the AT characteristic of radioresistant DNA synthesis. Southern blot analysis demonstrated that the 1B3 cell line contains at least three cosmids that appear to be integrated in tandem and coamplified [Kapp and Painter 1989, id.]. Transfer of cellular DNA containing those integrated cosmid sequences to AT5BIVA cells produced cell clones with radioresistance similar to that of 1B3 cells, indicating that a gene within the cosmids complements the defect in the AT-D group [Kapp and Painter 1989, id].

Functional compl mentation of that same cell line (AT5BIVA) has b en accomplished by microcell-mediated chromosome transfer from mouse-human hybrids [Lambert et al.

1991]. That study showed that the gene for AT-D was within a recombinant chromosome that contain d a human chromosome 11q23 fragment that was telomeric to the AT-A and AT-C linkage region. However, the report did not state whether that mouse-human hybrid also contained the chromosome 11q23 region telomeric to THY1.

The inventors hereof cloned DNA from a fragment of an AT gene for complementation group D. That DNA was of value as a probe to find and clone an entire AT gene for complementation group D, that is, an ATDC gene, and to identify a region in 11q23 where the ATDC gene is located. Prior to the cloning described herein, basic research and clinical work on AT had moved very slowly as there had been no specific gene recognized for AT. Further, there had been no simple biochemical or laboratory test to identify AT patients and/or AT heterozygotes accurately or to classify patients into various complementation groups. The instant invention provides a clear direction for AT research and the means to identify mutations in ATDC genes. Identifying such mutations provides for methods to diagnose AT, preferably AT-D, and to detect AT heterozygotes, preferably AT-D heterozygotes. Detection of AT heterozygotes is important because they have been reported to have an increased risk of cancer in response to treatment with ionizing radiation [Swift et al., N. Engl. J. Med., 325: 1831-1836 (1991)].

SUMMARY OF THE INVENTION

Herein disclosed is an AT gene for complementation group D, the ATDC gene. Fragments of said gene are useful as nucleic acid probes and as polymerase chain reaction (PCR) primers. One embodiment of said ATDC gene comprises the human DNA in cosmids K1 and 4-1 which were deposited at the American Type Culture Collection [ATCC; Rockville, MD (USA)] on June 16, 1992, respectively under ATCC Nos. 75250 and 75251. This invention thus concerns, in one asp ct, compositions comprising an isolated ATDC gene or one or more fragments thereof; the nucleotide sequence for said gene or fragment(s) thereof is or are substantially complementary to the

nucleotide sequence of the ATDC gene contained in cosmid K1 and/or cosmid 4-1, or to fragments of said nucleotide sequence contained within either or both of said cosmids.

Further disclosed is cDNA from said ATDC gene, preferably a 3 kilobase (kb) cDNA and/or fragments thereof, the entire nucleotide sequence for which is shown in Figures 4A-4C [SEQ. ID. NO.: 1]. Still further disclosed are AT proteins and/or polypeptides translated from said cDNA, preferably from said 3 kb cDNA. The amino acid sequence for a preferred AT protein is shown in Figures 5A-5D [SEQ. ID. NO.: 3]. Portions of said amino acid sequence are included in this invention as well as homologous variants of said amino acid sequence and portions of said homologous variants. Within the scope of this invention are AT proteins and/or polypeptides, preferably ATDC proteins and/or polypeptides, produced recombinantly, chemically and/or biologically, wherein said proteins and/or polypeptides are in a substantially pure form.

In one facet, this invention concerns an isolated nucleic acid sequence encoding a human AT protein/polypeptide wherein the sequence is substantially complementary, that is, hybridizable under standard stringency conditions, to a nucleic acid sequence encoding the amino acid sequence depicted in Figures 5A-5D, or at least a portion of said amino acid sequence. Representative nucleic acid sequences encoding such a human AT protein/polypeptide are those nucleic acid sequences shown in Figures 4A-4C [SEQ. ID. NO.: 1] and in Figures 5A-5D [SEQ. ID. NO.: 2].

Still further, this invention concerns recombinant nucleic acid molecules comprising a genomic DNA sequence or a cDNA sequence that encodes an AT protein or polypeptide, wherein said DNA or cDNA sequence is operatively linked to an expression control sequence in said nucleic acid molecule. Exemplary embodiments of said recombinant nucleic acid molecules include thos wherein the genomic DNA sequence is selected from the group consisting of the DNA sequence of the ATDC gene contained in cosmid K1 and cosmid 4-1, DNA sequences substantially complementary to the DNA sequence contained in those cosmids, fragments of the DNA sequence contained in

those cosmids, and fragments of DNA sequences that are substantially complementary to the DNA sequence contained in said cosmids. Representative embodiments of said recombinant molecules also include those wherein the cDNA sequence is selected from the group consisting of the 3 kilobase (kb) cDNA shown in Figures 4A-4C [SEQ. ID. NO.: 1], DNA sequences substantially complementary to said 3 kb cDNA, the cDNA sequence shown in Figures 5A-5D [SEQ. ID. NO.: 2], and DNA sequences substantially complementary to the cDNA sequence shown in Figures 5A-5D.

Still further disclosed are polymerase chain reaction (PCR) primers for amplifying each of the 9 exons of said 3 kb cDNA. Said primers are delineated in Figure 7 [SEQ. ID. NOS.: 4-31], and the 14 PCR fragments amplified by said primers are shown in Figures 8A-8N [SEQ ID. NOS.: 32-45].

Said ATDC gene, fragments thereof and/or the related cDNA are useful as follows: 1) to identify AT proteins and polypeptides as well as homologs or near-homologs thereto, preferably of the AT complementation group D; 2) to identify mutations in ATDC genes leading to AT-D; 3) to identify various mRNAs transcribed from ATDC genes in various AT cell lines and from different human tissues; 4) to provide the means to construct probes to test for persons with AT and that are AT heterozygotes; 5) and to elucidate the mechanisms by which AT mutations lead to radiosensitivity, immunodeficiences and ataxia.

For example, fragments of the ATDC gene as contained in the deposited cosmids K1 and 4-1, or fragments thereof, or the 3 kb cDNA as shown in Figures 4A-4C and/or fragments thereof, or the PCR primers for each of the nine exons, can be used as probes to identify and isolate ATDC genes from various AT patients. The nucleotide sequences of the ATDC gene from persons with AT and from those without AT can be compared, and the mutation(s) responsible for AT pleiotropic abnormalities can be identified. Once the AT mutation(s) is or are identified, nucleic acid probes, preferably DNA probes, or PCR primers, can be constructed that span the mutated region(s). Such probes or PCR primers will provide the basis for the

development of tests, hybridization or PCR assays, to identify people with AT and heterozygote carriers of the mutated AT gene. PCR assays are preferred.

The sequence of the ATDC gene and/or fragments thereof and the resulting probes should allow for research to progress on the mechanism(s) underlying the pleiotropic abnormalities of AT and investigations into the reported high rates of some types of cancers in AT heterozygotes.

A representative method of detecting mutations in an ATDC gene according to this invention comprises the steps of:

amplifying one or more fragment(s) of said gene and of an ATDC gene known to be normal, by the polymerase chain reaction (PCR) with the same or substantially the same PCR primers; and

determining whether said ATDC gene contains any mutations by comparing the PCR products of the amplification of said gene with those from the amplification of the normal gene, and detecting differences between the PCR products associated with mutations. Preferred PCR primers for that representative method are selected from the group consisting of the primers shown in Figure 7 [SEQ. ID. NOS.: 4-31] and nucleic acid sequences substantially complementary thereto. Differences detected by said method are, for example, differences in size and in base sequence. Preferred methods of detecting mutations according to this invention include the use of a PCR-single-stranded conformation polymorphism assay or denaturing gradient electrophoretic assay.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A illustrates the structure of cosmid clones K1 and K41. The locations of the human DNA (thinner black lines) and integrated pCV108 cosmid sequences (undarkened bars) are shown. The neo gene, with an SV40 promoter and bifunctional termination sequences are positioned as shown. The darkened bars indicate restriction fragments in the K1 and K41 cosmids that contain sequences complementary to cDNA from a HeLa cDNA library. The locations of the four EcoRI (E) fragments combined as probes for identification of cDNA clones

are shown, as are the locations of the r striction-enzymes sites for BglII (B), SmaI (Sm), SstI (Ss), XbaI (Xb), and XhoI (Xh).

Figure 1B illustrates the structure of the full-length 3.0 kilobase (kb) cDNA.

Figure 2A illustrates the structure of the 4-1 and 3-1 cosmids containing the 3' portion of the ATDC gene (the ataxia-telangiectasia complementation group D gene).

Figure 2B illustrates the structure of the intact ATDC gene, as reconstructed from the sequences in the K1 (left) and 4-1 (right) cosmids. The symbols for the DNA sequences and restriction sites are as defined in the description of Figures 1A-1B, supra.

Figure 3 provides a graph of survival as a function of X-ray dose for the following cell lines: AT5BIVA (indicated by blackened circle); 1B3 (indicated by unblackened circles); and C6 (indicated by blackened triangles).

Figures 4A-4C show the nucleotide sequence for the full-length 3 kb cDNA [SEQ. ID. NO.: 1], the restriction map for which is shown in Figure 1B.

Figures 5A-5D provide the translated open reading frame nucleotide sequence [SEQ. ID. No.: 2] for the 3 kb cDNA. [The amino acid sequence is SEQ. ID. NO. 3.]

Figure 6 schematically shows the nine exons of the 3 kb cDNA. Fourteen PCR fragments are delineated wherein the arrows indicate the positions of the primers. The primers are shown in Figure 7, and the sequences of the PCR fragments in Figures 8A-8N. The open box indicates the untranslated region whereas the hatched boxes indicate the region of the alpha helix.

Figure 7 provides the nucleotide sequences for the PCR primers [SEQ. ID. NOS.: 4-31] for the 3 kb cDNA, the placement of which is shown schematically in Figure 6 and specifically in Figures 8A-8N.

Figures 8A-8N provide the nucleotide sequences [SEQ. ID. NOS. 32-45] for the PCR fragments of the 3 kb cDNA schematically indicated in Figure 6 as well as the placement of the PCR primers listed in Figure 7.

Nucleotide and Amino Acid Sequence Symbols

The following symbols are used to represent nucleotides in the figures herein:

Base	Symbol
adenine	A
cytosine	C
guanine	G
thymine	T
uracil	U

The symbol Y represents cytosine or thymine/uracil; whereas the symbol N represents adenine or cytosine or guanine or thymine/aracil according to the standard convention.

It is understood that because of the degeneracy of the genetic code, that is, that more than one codon will code for one amino acid [for example, the codons TTA, TTG, CTT, CTC, CTA and CTG each code for the amino acid leucine (leu)], that variations of the nucleotide sequence in, for example, Figures 4A-4C, wherein one codon is substituted for another, would produce a substantially equivalent protein or polypeptide according to this invention. All such variations in the nucleotide sequence of the 3 kb cDNA are included within the scope of this invention.

It is further understood that the nucleotide sequence herein described and shown in Figures 4A-4C represent only the precise structure of the cDNA nucleotide sequence isolated according to this invention. It is expected that slightly modified nucleotide sequences will be found or can be modified by techniques known in the art to code for substantially similar AT proteins and polypeptides, for example, those having similar epitopes, and such nucleotide sequences and proteins/polypeptides are considered to be equivalents for the purpose of this invention. DNA having equivalent codons is considered within the scope of the invention, as are synthetic DNA sequences that encode proteins/polypeptides homologous or substantially homologous to AT proteins/polypeptides, as well as those sequences but for the degeneracy of the genetic code would hybridize to said

3 kb cDNA nucleotide sequence. Modifications and variations of DNA sequences as indicated herein are considered to result in sequences that are substantially the same as the ATDC sequences and fragments thereof herein.

There are twenty main amino acids, each of which is specified by a different arrangement of three adjacent DNA nucleotides (triplet code or codon), and which are linked together in a specific order to form a characteristic protein. A three-letter convention is used herein to identify said amino acids, as, for example, in Figures 5A-5D, as follows:

Amino acid name	Symbol
Alanine	Ala
Arginine	Arg
Asparagine	Asn
Aspartic Acid	Asp
Cysteine	Cys
Glutamic Acid	Glu
Glutamine	Gln
Glycine	Gly
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Serine	Ser
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr
Valine	Val

Abbreviations

The following abbreviations are used herein.

AT - ataxia-telangiectasia

AT-A - ataxia-telangiectasia complementation group A

AT-B - ataxia-telangiectasia complementation group B

AT-C	-	ataxia-telangiectasia complementation group C
AT-D	-	ataxia-telangiectasia complementation group D
ATCC	-	American Type Culture Collection
ATDC gene	-	AT-D complementing gene
B	-	BglII
DAPI	-	diamino-2-phenylindole
E	-	EcoRI
FITC	-	fluorescein isothiocyanate
Gy	-	gray; unit of absorbed radiation according to Systeme International d'Unites
kb	-	kilobase
M	-	molar
min	-	minute
ml	-	milliliter
PCR	-	polymerase chain reaction
Sm	-	SmaI
Ss	-	SstI
SV	-	simian virus
μg	-	micrograms
Xb	-	XbaI
Xh	-	XhoI

DETAILED DESCRIPTION

Herein disclosed is a new gene, an AT gene, more specifically the ATDC gene. That gene was isolated by complementation of ionizing radiation sensitivity in an AT cell line from complementation group D. Claimed herein is said ATDC gene and nucleotide sequences that are substantially complementary to said gene or fragments thereof. Strong evidence is provided herein to indicate that a defect in said gene is responsible for radiosensitivity in AT cells of complementation group D.

To isolate that gene, the radiosensitive AT cells of the AT5BIVA cell line were first transfected with a human cosmid library containing a selectable neo gene, followed by selection with both the antibiotic G418 and ionizing radiation to isolate cell clones containing human/cosmid DNA that complements the defective gene. A cell clone (1B3) that

demonstrates partial recovery of resistance to ionizing radiation was isolated. That clone was found to contain three integrated human/cosmid sequences.

The integrated human/cosmid sequences were rescued by a process which first comprised the construction of a cosmid library from the 1B3 cell DNA and then the selection with kanamycin for clones containing the neo gene. The selected cosmid clones were then used as chromosome-specific painting probes [Pinkel et al., PNAS (USA), 85: 9138-9142 (Dec. 1988); Gray et al., European Patent Application 430,402 (published June 5, 1991); and Ward et al., WO 90/05789 (published May 31, 1990)] for in situ hybridization to chromosomes from normal human lymphocytes. Two related cosmid clones (K1 and K41) were found to contain human sequences that originated from chromosomal region 11q23. As indicated in the Background above, the 11q23 region has been previously determined by genetic linkage analysis to be the location of the AT gene(s) from three separate complementation groups.

Restriction site mapping of the cosmid clones showed that they contain over 36 kilobases (kb) of human DNA located to one side of an integrated cosmid sequence in cell clone 1B3. Southern blot analysis with total human DNA revealed that most of the 36 kb was single-copy DNA. A cDNA library was screened with parts of said single-copy DNA as probes, and two clones that hybridized were isolated, indicating that the ATDC gene is transcribed in relative abundance. Transfection of one of the cosmids (K1) back in the radiosensitive parental AT cell line (AT5BIVA) resulted in the isolation of one cell clone that exhibited radio-resistance similar to that for 1B3.

Sequence analysis has shown that ATDC is a previously unidentified gene. Although the ATDC gene is a single-copy gene, five mRNAs have been detected in a range of from about 1.8 kilobases (kb) to about 5.7 kb. The variation in size of the mRNAs may result from differences at the 5' end, indicating that the ATDC gene is a complex gene that can code for several different proteins. The ATDC gene may therefore have multiple functions, consistent with the pleiotropic abnormalities seen in individuals with AT.

Fine mapping of the location of the ATDC gene, as described below, by means of radiation hybrid cell lines, demonstrated that the ATDC gene is located just telomeric to the THY1 marker, that is, in the region that had been indicated by linkage analysis to contain the AT gene from complementation group D.

The ATDC gene and/or fragments thereof and/or cDNA therefrom may be used as probes to identify homologous genes from other AT complementation groups. Linkage analysis has demonstrated that the AT gene(s) for the various complementation groups are clustered together, and thus it is possible that they are distantly related. Chromosome 11-specific libraries, preferably chromosome 11q23-specific libraries, and still more preferably chromosome 11q23-specific cosmid libraries, can be screened to identify clones that cross hybridize with ATDC.

Further, mouse cDNA libraries can be screened with ATDC probes for a related mouse gene. Isolation of a mouse homolog would allow for the development of a transgenic mouse AT model.

The probes and assays enabled by this invention will be useful in proving that a defect in the ATDC gene is responsible for AT-D. Proof required would be (1) the demonstration of genetic rearrangements, such as, deletions, amplifications, translocations, inversions, point mutations among others, in the ATDC gene in AT-D cells, and/or (2) functional complementation of radiosensitivity in AT-D cells after transfection with an appropriate vector, preferably a cosmid vector containing the gene or a fragment thereof, or a cDNA therefrom in an expression vector. That there are no detectable rearrangements within the ATDC gene in the AT5BIVA cell line suggests a small rearrangement or point mutation, as observed in other human genetic diseases [Gibbs et al., Genomics, 7: 235-244 (1990); Groden et al., Cell, 66: 589-600 (1991)]. DNA from other AT cell lines from unknown complementation groups also showed no identifiable alterations in the ATDC gene. Preferably sequence analysis of the ATDC gene in cells obtained exclusively from AT-D individuals will

be used to determine whether mutations in the gene are associated with AT.

Assays to Detect Mutations in the ATDC Gene

An important utility of the instant invention is to detect the mutations that cause defect(s) in the ATDC gene resulting in the phenotypic symptoms of AT-D. To detect relatively large genetic rearrangments, hybridization tests, preferably Southern or Northern assays, can be used. To detect relatively small genetic rearrangements, as for example, small deletions or amplifications, or point mutations, assays incorporating PCR are preferably used.

Preferred methods of identifying mutations within the ATDC gene comprise assays wherein PCR is used. The mechanics of PCR are described in Saiki et al., Science, 230: 1350 (1985) and U.S. Patent Nos. 4,800,159 (issued January 24, 1989), 4,683,195 and 4,683,202 (both of the latter issued July 28, 1987). Preferably, such a PCR assay amplifies and analyses cellular DNA, such that mutations within splice sites as well as within coding regions can be detected when appropriate primers are used. However, mRNA could also be isolated, and cDNA prepared therefrom. Appropriate PCR primers could then be used to amplify the cDNA, and the cDNA PCR products from normal and AT cells could then be compared.

An exemplary PCR assay is that wherein the PCR primers listed in Figure 7 [SEQ. ID. NOS.: 4-31] are used. In such an assay, cellular DNA from people with AT and without AT is isolated and amplified with the PCR primers. The PCR products from the normal and AT cells are compared, preferably initially upon a sizing gel. Changes in size indicative of certain genetic rearrangements are thus determined. If no changes are found in the size of the fragments, then further comparisons can be made to detect genetic rearrangements that would not be evident in such a size comparison, for example, deletions of a few base pairs or point mutations. To effect such further comparisons, preferred methods include the use of a PCR-single-strand conformation polymorphism (PCR-SSCP) assay or a denaturing gradient gel electrophoretic assay. The PCR-

SSCP method is described in a revi w article by Kenshi Hayashi entitled "PCR-SSCP: A Simple and Sensitive Method for Detection of Mutations in the Genomic DNA" in PCR Methods and Applications, 1: 34-38 (1991). Denaturing gradient gel electrophoresis is described in Myers et al., "Detection and Localization of Single Base Changes by Denaturing Gradient Gel Electrophoresis," Methods in Enzymology, 155: 501-527 (1987).

Such assays, as hybridization and PCR assays, preferably PCR assays including the PCR-SSCP or denaturing gradient gel electrophoretic assays, as applied in the context of this invention may be used to screen people for AT heterozygosity, which can be a significant risk factor in regard, for example, to ionizing radiation, and for prenatal testing for and diagnosis of AT, among other uses.

AT Proteins and/or Polypeptides

The phrase "AT proteins and/or polypeptides" is herein defined to mean proteins and/or polypeptides encoded by an ATDC gene and/or fragments thereof. An exemplary and preferred AT protein is that for which the amino acid sequence is shown in Figures 5A-5D [SEQ. ID. NO.: 3].

A "polypeptide" is a chain of amino acids covalently bound by peptide linkages and is herein considered to be composed of 50 or less amino acids. A "protein" is herein defined to be a polypeptide composed of more than 50 amino acids.

It will be appreciated that the amino acid sequence of AT proteins and polypeptides can be modified by genetic techniques. One or more amino acids can be deleted or substituted. Such amino acid changes may not cause any measurable change in the biological activity of the protein or polypeptide and result in proteins or polypeptides which are within the scope of this invention.

The AT proteins and polypeptides of this invention can be prepared in a variety of ways according to this invention, for example, recombinantly, synthetically or otherwise biologically, that is, by cleaving longer proteins

and polypeptides enzymatically and/or chemically. A preferred method to prepare AT proteins is by recombinant means.

A representative method to prepare the AT protein as shown in Figures 5A-5D or fragments thereof would be to insert the appropriate fragment of the 3 kb cDNA pictured in Figures 4A-4C into an appropriate expression vector. A wide variety of host-cloning vector combinations may be usefully employed in cloning the ATDC DNA isolated as described herein. For example, useful cloning vehicles may include chromosomal, nonchromosomal and synthetic DNA sequences such as various known bacterial plasmids such as pBR322, other E. coli plasmids and their derivatives and wider host range plasmids such as RP4, phage DNA such as the numerous derivatives of phage lambda, e.g., NB989 and vectors derived from combinations of plasmids and phage DNAs such as plasmids which have been modified to employ phage DNA expression control sequences.

Useful hosts may be eukaryotic or prokaryotic and include bacterial hosts such as E. coli and other bacterial strains, yeasts and other fungi, animal or plant hosts such as animal or plant cells in culture, insect cells and other hosts. Of course, not all hosts may be equally efficient. The particular selection of host-cloning vehicle combination may be made by those of skill in the art after due consideration of the principles set forth herein without departing from the scope of this invention.

The particular site chosen for insertion of the selected DNA fragment into the cloning vehicle to form a recombinant DNA molecule is determined by a variety of factors. These include size and structure of the protein or polypeptide to be expressed, susceptibility of the desired protein or polypeptide to endoenzymatic degradation by the host cell components and contamination by its proteins, expression characteristics such as the location of start and stop codons, and other factors recognized by those of skill in the art.

The recombinant nucleic acid molecule containing the ATDC gene, fragment thereof, or cDNA thereform, may be

employed to transform a host so as to permit that host (transformant) to express the structural gene or fragment thereof and to produce the protein or polypeptide for which the hybrid DNA codes. The recombinant nucleic acid molecule may also be employed to transform a host so as to permit that host on replication to produce additional recombinant nucleic acid molecules as a source of ATDC DNA and fragments thereof. The selection of an appropriate host for either of these uses is controlled by a number of factors recognized in the art. These include, for example, compatibility with the chosen vector, toxicity of the co-products, ease of recovery of the desired protein or polypeptide, expression characteristics, biosafety and costs.

Where the host cell is a procaryote such as E. coli, competent cells which are capable of DNA uptake are prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method by well known procedures. Transformation can also be performed after forming a protoplast of the host cell.

Where the host used is an eucaryote, transfection method of DNA as calcium phosphate-precipitate, conventional mechanical procedures such as microinjection, insertion of a plasmid encapsulated in red blood cell hosts or in liposomes, treatment of cells with agents such as lysophosphatidyl-choline or use of virus vectors, or the like may be used.

The level of production of protein or polypeptide is governed by two major factors: the number of copies of its gene or DNA sequence encoding for it within the cell and the efficiency with which these gene and sequence copies are transcribed and translated. Efficiencies of transcription and translation (which together comprise expression) are in turn dependent upon nucleotide sequences, normally situated ahead of the desired coding sequence. These nucleotide sequences or expression control sequences define, inter alia, the location at which RNA polymerase interacts to initiate transcription (the promoter sequence) and at which ribosomes bind and interact with the mRNA (the product of transcription) to initiate translation. Not all such expression control

sequences function with equal efficiency. It is thus of advantage to separate the specific coding sequences for the desired protein from their adjacent nucleotide sequences and fuse them instead to known expression control sequences so as to favor higher levels of expression. This having been achieved, the newly engineered DNA fragment may be inserted into a multicopy plasmid or a bacteriophage derivative in order to increase the number of gene or sequence copies within the cell and thereby further improve the yield of expressed protein.

Several expression control sequences may be employed. These include the operator, promoter and ribosome binding and interaction sequences (including sequences such as the Shine-Dalgarno sequences) of the lactose operon of E. coli ("the lac system"), the corresponding sequences of the tryptophan synthetase system of E. coli ("the trp system"), a fusion of the trp and lac promoter ("the tac system"), the major operator and promoter regions of phage lambda (O_LP_L and O_RP_R,), and the control region of the phage fd coat protein. DNA fragments containing these sequences are excised by cleavage with restriction enzymes from the DNA isolated from transducing phages that carry the lac or trp operons, or from the DNA of phage lambda or fd. These fragments are then manipulated in order to obtain a limited population of molecules such that the essential controlling sequences can be joined very close to, or in juxtaposition with, the initiation codon of the coding sequence.

The fusion product is then inserted into a cloning vehicle for transformation of the appropriate hosts and the level of antigen production is measured. Cells giving the most efficient expression may be thus selected. Alternatively, cloning vechicles carrying the lac, trp or lambda P_L control system attached to an initiation codon may be employed and fused to a fragment containing a sequence coding for an AT protein or polypeptide such that the gene or sequence is correctly translated from the initiation codon of the cloning vehicle.

The phrase "recombinant nucleic acid molecule" is herein defined to mean a hybrid nucleotide sequence comprising at least two nucleotide sequences, the first sequence not normally being found together in nature with the second.

The phrase "expression control sequence" is herein defined to mean a DNA sequence of nucleotides that controls and regulates expression of structural genes when operatively linked to those genes.

Synthetic and Biologic Production of AT Proteins and Polypeptides

AT proteins and polypeptides of this invention may be prepared not only by recombinant means but also by synthetic and by other biologic means. Exemplary of other biologic means to prepare the desired polypeptide or protein is to subject to selective proteolysis a longer AT polypeptide or protein containing the desired amino acid sequence; for example, the longer polypeptide or protein can be split with chemical reagents or with enzymes. Synthetic formation of the polypeptide or protein requires chemically synthesizing the desired chain of amino acids by methods well known in the art.

Chemical synthesis of a peptide is conventional in the art and can be accomplished, for example, by the Merrifield solid phase synthesis technique [Merrifield, J., Am. Chem. Soc., 85: 2149-2154 (1963); Kent et al., Synthetic Peptides in Biology and Medicine, 29 f.f. eds. Alitalo et al., (Elsevier Science Publishers 1985); and Haug, ABL, 40-47 (Jan/Feb. 1987)].

Techniques of chemical peptide synthesis include using automatic peptide synthesizers employing commercially available protected amino acids, for example, Biosearch [San Rafael, CA (USA)] Models 9500 and 9600; Applied Biosystems, Inc. [Foster City, CA (USA)] Model 430; Milligen [a division of Millipore Corp.; Bedford, MA (USA)] Model 9050; and Du Pont's RAMP (Rapid Automated Multiple Peptide Synthesis) [Du Pont Compass, Wilmington, DE (USA)].

The following materials and methods were used in the examples illustrating this invention.

Cell Lines

The simian virus (SV)40-transformed cell lines LM217 (normal) and AT2SF (unknown AT complementation group) were established by transfection of primary human fibroblasts with pSVori- plasmid [Murnane et al., Exp. Cell Res., 158: 119-126 (1985); Murnane et al., Mol. Cell Biol., 6: 549-558 (1986)].

The SV40-transformed fibroblast cell line AT3BISV (AT-A) was provided by A. M. R. Taylor (Birmingham, United Kingdom).

Cell line 1B3 was derived from the SV40-transformed fibroblast cell line AT5BIVA (GM5849) [Kapp and Painter 1989, supra], which was obtained from the NIGMS Human Genetic Mutant Cell Repository [Camden, NJ (USA)]. HeLa cells (Hela S3; ATCC CCL 2.2) were obtained from the American Type Culture Collection [ATCC; Rockville, MD (USA)].

Cosmid Library Construction

The cosmid library from 1B3 was constructed according to standard procedures [Sambrook et al., "Molecular Cloning: A Laboratory Manual", 2d ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; 1989)]. DNA from 1B3 was partially digested with MboI, and fragments of about 30 kilobases (kb) to about 50 kb were ligated to BamHI-digested pWE16 cosmid DNA [Stratagene, La Jolla, CA (USA); Wahl et al., PNAS, 84: 2160 (1987)]. Clones containing the neo (neomycin resistance) gene in the integrated pCV108 cosmid [Lau and Kan, PNAS, 80: 5225 (1983)] DNA originally used for transfection [Kapp and Painter (1989)] were selected by growth of the XL1-Blue bacteria (Stratagene) containing the cosmid library on agar plates with 50 μg kanamycin/ml. Restriction-enzyme mapping was accomplished by digestion with various combinations of enzymes and was confirmed by further mapping of the individual EcoRI fragments cloned into a Bluescript plasmid (Stratagene).

Cosmid and cDNA Library Screening

A commercially available HeLa cell cDNA library (Stratagene) within the bacteriophage vector ZAPII was

screened according to a method described elsewhere [Murnane, Mol. Cell Biol., 6: 549-558 (1986)] with a probe that consisted of cosmid fragments containing only nonrepetitive sequences. Positive cDNA clones were then rescued from ZAPII by in vivo excision of the Bluescript plasmid by using a protocol provided with the vector (Stratagene). A chromosome 11-specific cosmid library obtained from L. Deaven [Los Alamos National Laboratory; Los Alamos, NM (USA)] was screened by conventional methods (Sambrook et al. 1989, supra) with the use of a 3.0 kb cDNA as a probe (Figures 1B, 4A-4C and 8A-8N).

Southern and RNA Blot Analysis

Preparation of high-molecular-weight cellular DNA and Southern blot analysis were as described elsewhere (Murnane 1986, supra). mRNA was prepared with the use of an mRNA isolation kit [Fast Track^R; Invitrogen; San Diego, CA (USA)]. RNA agarose gel electrophoresis and RNA blot analysis were carried out according to standard procedures (Sambrook et al. 1989, supra).

Functional Complementation

DNA to be tested for complementation was introduced into AT5BIVA cells by calcium phosphate-mediated transfection (Murnane et al. 1985, supra). Before transfection, the cosmid clones were linearized with NotI, which cuts between the vector and human sequences. Cell clones containing the integrated DNA were selected by incubation with 400 μg G418/ml. X-ray survival was determined according to a method described elsewhere (Kapp and Painter 1989, supra) with or without feeder layers that consisted of HeLa cells exposed to 60 Gy (gray) of X-rays.

In Situ Hybridization

Metaphase spreads were prepared according to the method described by Yunis in Science, 191: 1268-1270 (1976). Slides were treated with 20 μg pepsin/ml (in 0.1 M HCl) for 10 min at 37°C before in situ hybridization. Hybridization conditions and staining procedures were modified from those of

Pinkel et al. [PNAS (USA), 83: 2934-2938 (1986)] and Trask et al. [Am. J. Hum. Genet., 48: 1-15 (1991)]. The in situ hybridization probe strategy specifically comprised the use of a dioxigenin-labeled K1 or K41 cosmid probe that was detected with FITC-conjugated antibodies, and a biotin-labeled chromosome 11-specific alpha-satellite DNA probe that was detected with Texas Red-conjugated avidin. The chromosome 11-specific alpha-satellite DNA probe was used as a marker in locating the origin of the cosmid probes to human chromosomal region 11q23. Chromosomes were counterstained with 4',6-diamino-2-phenylindole (DAPI).

Radiation Hybrid Mapping

The chromosomal map location of the ATDC gene was determined by a radiation hybrid mapping technique [Richard et al., Am. J. Hum. Genet., 49: 1189-1196 (1991)]. The order and distances between ATDC and the markers stromely-sin 1 (STMY1), CJ52.193 (D11S384), CJ52.77 (D11S424), CD3D, apolipoprotein (APO), THY1, D11S528, or ETS1 were established by a statistical analysis of the cosegregation of markers in 100 radiation hybrids, and the method of moments was used to determine the frequency of breakage between markers [Cox et al., Science, 250: 245-250 (1990)]. Because a single retention frequency was used in this analysis, and since each hybrid was scored for almost every marker, the likelihood for any marker order was estimated as the sum of the pairwise lod scores between markers. The map with the highest likelihood was defined as that map in which the sum of lod scores between adjacent loci was maximized. The polymerase chain reaction (PCR) with primers specific for the 3' end of the 3.0 kb cDNA [primers 9D-5 and 9D-3 (SEQ. ID. NOS.: 30 and 31) shown in Figure 7] was used to determine which of the radiation hybrids contained the ATDC gene. The conditions for PCR were the same as those used for mapping other markers on human chromosome 11q (Richard et al. 1991, supra).

The following examples are for purposes of illustration only and are not meant to limit the invention in any way.

Example 1

Isolation of Integrated Cosmid DNA

The integrated cosmid sequences from cell line 1B3 were isolated by construction of a cosmid library from 1B3 DNA, and then by using kanamycin to select bacteria that had cosmids containing the integrated neo gene. Cosmid clones isolated by this method were screened by in situ hybridization to identify cosmids containing human DNA from the chromosomal region 11q23, previously demonstrated to be the location of the gene(s) associated with three AT complementation groups. Two of the cosmid clones (K1 and K41) hybridized to chromosomal region 11q23. As indicated above, a dioxigenin-labeled K1 or K41 cosmid probe was localized to 11q23 in a chromosome-specific painting experiment wherein a chromosome 11-specific alpha-satellite DNA probe was used concurrently to identify the centromeric chromosome 11 region.

The rescue from the integration site in 1B3 of DNA that originated from the chromosomal region 11q23 is further strong evidence that a gene within the transfected DNA complements radiosensitivity in cells from AT-D. According to restriction-enzyme mapping, K1 and K41 contained overlapping sequences, consisting of both the integrated pCV108 cosmid and adjacent human DNA (Figure 1A).

To determine whether the cloned human DNA contained any expressed cellular genes, fragments of cosmid K1 were used as probes to screen a HeLa cell cDNA library. Four adjacent EcoRI fragments that contained only nonrepetitive cellular DNA (Figure 1A) were combined for this purpose. After hybridization to 1.2×10^6 cDNA clones, 40 positive plaques were identified. Restriction-enzyme mapping of nine of these cDNA clones demonstrated that they were all related and that the five largest (3.0 kb) were nearly identicial (Figure 1B). Partial sequence analysis of the ends of the 3.0-kb cDNAs has identified the 3' end by the presence of a tract of poly-A and suggests that they are full length because the 5' ends show two stop codons before the first methionine codon in the only complete open reading frame. [Full nucleotide sequence of 3 kb cDNA is shown in Figures 4A-4C.] That gene contained

within those cosmids is herein termed the "AT-D complementing (ATDC) gene."

The approximate position of the sequences complementary to the cDNA within the K1 cosmid was determined by Southern blot analysis. Southern blots of K1 cosmid DNA digested with various restriction enzymes were hybridized with either the full-length 3.0-kb cDNA or a fragment from the 5' end. By the location of the labeled restriction fragments, two separate regions of hybridization were identified (Figure 1A). Hybridization with the fragment from the 5' end of the cDNA indicated that the direction of transcription in the cosmid was from left to right (Figure 1A). Consistent with those results are the similarity of the restriction sites (XhoI, SstI, and SmaI) between the cDNA and cosmid clones (Figure 1), as well as partial sequence analysis, which also demonstrated that the region of hybridization to the left (Figure 1A) coded for the 5' end of the transcript.

Not all of the sequences found in the cDNA were contained within the K1 and K41 cosmids. This was evident from the absence of additional hybridization regions containing SstI and EcoRI restriction sites corresponding to those found within the cDNA and indicates that nearly half of the coding sequences within the cDNA were absent from these cosmids. The fact that the transfected pCV108 sequences were located adjacent to the ATDC gene in both the K1 and K41 cosmid clones shows that the truncated gene is the form integrated into cell line 1B3. The orientation of the integrated sequences placed the SV40 bidirectional transcriptional termination sequences, which are located adjacent to the neo gene in the pCV108 cosmid, down-stream from the truncated gene (Figure 1A). The form of the ATDC gene integrated into cell line 1B3 is therefore a functional transcriptional unit despite the absence of the 3' end.

The presence of only a portion of the ATDC gene in 1B3 can explain several properties of 1B3, namely: (a) radiosensitivity in 1B3 did not completely return to the level seen in normal cells [Kapp and Painter 1989, supra]; (b) 1B3 amplified the integrated DNA, whereas other independently

derived, related clones that did not amplify the integrated cosmid sequences failed to maintain radioresistance in culture [Kapp and Painter 1989]; and (c) 1B3 cells continued to show radioresistant DNA synthesis similar to that of the parental AT5BIVA cell line [Kapp and Painter 1989].

Example 2

Isolation of the 3' End of the ATDC Gene and Composite Mapping of the ATDC Gene

To obtain the missing portion of the ATDC gene, the cDNA was used as a probe to screen a human genomic chromosome 11 library. Two cosmid clones (3-1 and 4-1) were identified. Restriction mapping of these clones demonstrated that they contained overlapping regions (Figure 2A). With the use of the 3.0-kb cDNA (Figures 1B and 4A-4C) as a probe, Southern blot analysis revealed three separate regions of hybridization within the cosmid DNA (Figure 2A). One of these regions, which was contained within cosmid clone 4-1 but not within 3-1, overlapped with cosmid clones K1 and K41 (Figure 2A). That was determined by sequence analysis with primers derived from the cDNA sequence. Beyond the overlapping region (compare Figures 1A and 2A), the differences between K1 and 4-1 are apparently due to the rearrangements that occurred in K1 during transfection and that resulted in the loss of the 3' end of the gene. Cosmid clone 4-1 therefore contains the ATDC gene 3' portion that was missing from clones K1 and K41. A composite map derived from cosmid clones K1 and 4-1 indicates that the missing 3' portion of the ATDC gene is more than 30 kb in length (Figure 2B).

Southern blot analysis of human genomic DNA from normal cells with the 3.0-kb cDNA as a probe was consistent with the structure of the gene predicted from restriction-enzyme mapping analysis of the cosmid clones (Figure 2B). Bands detected after digestion of human DNA with BglII (1.8, 4.5, 8.0, and 15 kb), EcoRI (4.0, 6.0, and 20kb), and XbaI (9.0, 12, and 23kb) all corresponded to the expected fragments. [Lambda HindIII restriction fragments were used as markers.] The 1.8-kb BglII fragment contains only a small

number of complementary sequences and is difficult to detect. The absence of additional bands demonstrates that no other genes complementary to the ATDC gen ar present in the human genome. Southern blot analysis of DNA isolated from the parental SV40-transformed AT fibroblast cell line, AT5BIVA, as well as two other SV40-transformed AT fibroblast cell lines (AT3BISV, AT-A; AT2SF, unknown complementation group) gave results identical to those from the normal cell DNA.

Example 3

Characterization of mRNA Transcripts

Although ATDC is a single-copy gene, it produces several mRNAs of varous sizes, apparently because of alternate processing of RNA transcripts. RNA blot analysis of poly-A-selected mRNA isolated from an SV40-transformed fibroblast cell line (LM217) demonstrated two mRNA transcripts (5.7 and 4.7 kb) that hybridized to the 3.0-kb cDNA probe. In contrast, mRNA isolated from HeLa cells upon RNA blot analysis lacked the largest mRNA transcript (which was seen more clearly on shorter exposures), and instead contained at least two others (3.0 and 1.8 kb). In HeLa cells, the 3.0-kb mRNA transcript was the most abundant, consistent with the fact that the cDNA for this mRNA was the only one identified in the HeLa cell library (Figure 1B).

Two SV40-transformed AT fibroblast cell lines (AT3BISV and AT5BIVA) were shown upon RNA blot analysis to contain various amounts of the mRNA transcripts seen in the LM217 and HeLa cells. In contrast, the 1B3 cell line appeared identical to the SV40-transformed normal line LM217, in size of mRNAs. The significance of the differences between the mRNA observed in the AT3BISV and AT5BIVA cell lines and that seen in LM217 or 1B3 is not known. No additional mRNA transcript from the truncated ATDC gene was evident in 1B3, indicating that it is either below the level of detection or is similar in size to one of the mRNAs transcribed from the endogenous gene.

The production of multiple mRNAs has been observed with other mammalian genes and can result from multiple

promoters, alternate splicing, and/or alternat poly-A addition sites [Leff t al., Ann. Rev. Biochem., 55: 1091-1117 (1986)]. The presence of different mRNAs in epithelial (HeLa) and fibroblast (LM217, 1B3, AT5BIVA, and AT3BISV) cell lines, as shown by RNA analysis of poly-A-selected mRNA, could indicate tissue-specific processing of ATDC mRNA [Leff et al., 1986, id.] which would be consistent with the pleiotropic characteristics of AT. Because the 3.0-kb mRNA that produced the cDNA that was isolated from HeLa cells is not found in the LM217 cell line, functional complementation of radioresistance may require the cDNA from the 4.7-kb mRNA, which is present in both HeLa and LM217.

Example 4

Functional Complementation

After transfection of AT5BIVA cells with the K1 cosmid, 50 G418-resistant clones were tested for sensitivity to X-rays. Three clones showed increased radioresistance; one of them (C6) had about the same radioresistance as did the 1B3 cell line (Figure 3).

Thus, these initial functional complementation studies achieved a partial restoration of radioresistance in the AT5BIVA cell line as shown in Figure 3. The absence of full complementation with the K1 cosmid could be due to several factors. The large size of the ATDC gene may make complementation difficult because of the problems associated with integrating large intact DNA fragments into human cells [Colbere-Garapin et al., Gene, 50: 279-288 (1986); Hoeijmakers et al., Exp. Cell Res., 169: 111-119 (1987); Mayne et al., Gene, 66: 65-76 (1988) (erratum: Gene, 83: 395 (1989)]. Furthermore, because the ATDC gene produces mRNAs larger than the 3.0-kb mRNA characterized herein, it is unclear whether the K1 cosmid contains all of the coding sequences transfected into 1B3.

Example 5

Fine Mapping of the Chromosomal Location of the ATDC Gene

Radiation hybrids that contained mapped fragments of human chromosome 11 (Richard et al. 1991, supra) were used to determine the location of the ATDC gene. The position of the ATDC gene relative to known markers within 11q23 was established by using PCR to identify which of the radiation hybrids contained the gene. Statistical analysis of 100 radiation hybrid cell lines showed that the ATDC gene is closely linked to THY1 and D11S528, with lod scores of 12.2 and 17, respectively (Table 1). The order 11cen-APO-CD3D-THY1-ATDC-11qtel was 1,000 times more likely than the order 11cen-APO-CD3D-ATDC-THY1-11qtel which places the ATDC gene centromeric to THY1.

Thus, the ATDC gene lies outside the linkage region predicted to contain the AT gene(s) for complementation groups A and C, indicating a separate locus for the complementation group D gene. The results described herein clearly demonstrate that the ATDC gene is not linked to the centromeric (STMY1) and telomeric (D11S424) flanking markers for the AT-A and AT-C gene(s). However, the evidence that the ATDC gene isolated from 1B3 is telomeric to THY1 is consistent with the evidence for an additional AT gene within that region. It appears from the evidence that several genes within the 11q23 region are associated with AT; the lack of hybridization of ATDC to other human DNA sequences suggests that they are not closely related.

Table 1

Radiation Hybrid Two-Point Mapping Analysis of ATDC and Various Chromosome 11 Loci

Marker A	Marker B	cR_{9000}[a]	LOD[b]	No. of Clones Observed[c] + +	+ -	- +	- -	Total
ATDC	STMY1 ...		.5	7	13	15	64	99
ATDC	D11S384 .		1.4	9	11	14	65	99
ATDC	D11S424 .		1.0	8	12	13	66	99
ATDC	APO	81	3.1	10	10	8	71	99
ATDC	CD3D	37	7.7	13	7	3	76	99
ATDC	THY1	17	12.2	15	5	0	79	99
ATDC	D11S528 .	6	17.0	18	2	0	79	99
ATDC	ETS1		2.0	9	11	10	69	99
APO	CD3D	28	9.5	14	5	3	78	100
APO	THY1	50	5.3	11	8	5	76	100
CD3D	THY1	17	11.7	14	3	2	81	100

[a] Represents distances (in centiRays), which are shown only for linked markers (LOD > 3).

[b] Likelihood that two markers are linked.

[c] Number of hybrids that retain both markers A and B (+ +), marker A but not marker B (+ -), marker B but not marker A (- +), or neither marker A nor B (- -); total number of hybrids analyzed is also shown.

The materials listed below were deposited with the American Type Culture Collection (ATCC) in Rockville, MD (USA). The deposits were made under the provisions of the Budapest Treaty on the International Recognition of Deposited Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty). Maintenance of a viable culture is assured for thirty years from the date of deposit. The organism will be made available by the ATCC

under the terms of the Budapest Treaty, and subject to an agreement between the Applicants and the ATCC which assures unrestricted availability upon issuance of the pertinent U.S. Patent. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any Government in accordance with its patent laws.

Cosmid	Deposit Date	ATCC #
K1	June 16, 1992	75250
4-1	June 16, 1992	75251

The description of the foregoing embodiments of the invention have been presented for purposes of illustration and description. They are not intented to be exhaustive or to limit the invention to the precise form disclosed, and obviously many modifications and variations are possible in light of the above teachings. The embodiments were chosen and described in order to explain the principles of the invention and its practical application to enable thereby others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

All references cited herein are hereby incorporated by reference.

CLAIMS

We claim:

1. A composition comprising an isolated gene for ataxia-telangiectasia complementation group D (ATDC) or a fragment thereof, wherein the nucleotide sequence for said gene or for said fragment is the nucleotide sequence of the ATDC gene or a fragment of that ATDC gene that is contained in cosmid K1 and cosmid 4-1, deposited at the American Type Culture Collection (ATCC) under ATCC Nos. 75250 and 75251, respectively; or is substantially complementary to that ATDC gene sequence contained in said cosmids or to fragments of said ATDC gene sequence.

2. A composition comprising cosmid K1 and/or cosmid 4-1 deposited at the American Type Culture Collection (ATCC) under ATCC Nos. 75250 and 75251, respectively.

3. A composition comprising an isolated nucleic acid sequence encoding a human AT protein/polypeptide selected from the group consisting of: the cDNA sequence shown in Figures 4A-4C [SEQ. ID. NO.: 1], sequences substantially complementary to said cDNA sequence [SEQ. ID. NO.: 1], fragments of said cDNA sequence [SEQ. ID. NO.: 1], and fragments that are substantially complementary to fragments of said cDNA sequence [SEQ. ID. NO.: 1].

4. A composition according to Claim 3 wherein said one or more fragments of said cDNA sequence [SEQ. ID. NO.: 1] are the fragments shown in Figures 8A-8N [SEQ. ID. NOS.: 32-45], and the fragments that are substantially compl mentary to fragments of said cDNA sequence [SEQ. ID. NO.: 1] are nucleic acid sequences that are substantially complementary to the fragments shown in Figures 8A-8N [SEQ. ID. NOS.: 32-45].

5. A composition comprising polymerase chain reaction (PCR) primers selected from the group consisting of the primers shown in Figure 7 [SEQ. ID. NOS.: 4-31] and nucleic acid sequences substantially complementary thereto.

6. A composition comprising a protein or a polypeptide encoded by an ATDC gene or fragment thereof according to Claim 1.

7. A composition comprising an ataxia-telangiectasia (AT) protein or polypeptide having an amino acid sequence selected from the group consisting of: the amino acid sequence shown in Figures 5A-5D [SEQ. ID. NO.: 3]; a portion of the amino acid sequence shown in Figures 5A-5D; amino acid sequences which are a homologous variants of the amino acid sequence shown in Figures 5A-5D; and portions of amino acid sequences which are homologous variants of the amino acid sequence shown in Figures 5A-5D.

8. A recombinant nucleic acid molecule comprising a genomic DNA or cDNA sequence that encodes an AT protein or polypeptide according to Claim 7, wherein said genomic DNA or cDNA sequence is operatively linked to an expression control sequence in said nucleic acid molecule.

9. The recombinant nucleic acid molecule according to Claim 8 wherein said genomic DNA sequence is selected from the group consisting of: the DNA sequence of the ATDC gene contained in cosmid K1 and cosmid 4-1, deposited at the American Type Culture Collection (ATCC) under ATCC Nos. 75250 and 75251, respectively; DNA sequences substantially complementary to said DNA sequence contained in said cosmids; fragments of said DNA sequence contained in said cosmids; and DNA sequences substantially compl mentary to fragments of the DNA sequence contained in said cosmids.

10. The recombinant nucleic acid molecule according to Claim 10 wherein said cDNA sequence is selected from the

group consisting of: the 3 kilobase (kb) cDNA shown in Figures 4A-4C [SEQ. ID. NO.: 1]; DNA sequences substantially complementary to said 3 kb cDNA; the cDNA sequence shown in Figures 5A-5D [SEQ. ID. NO.: 2]; and DNA sequences substantially complementary to said cDNA sequence shown in Figures 5A-5D.

11. A method of detecting mutations in an ATDC gene comprising the steps of:

amplifying one or more fragment(s) of said gene and of an ATDC gene known to be normal, by the polymerase chain reaction (PCR) with the same or substantially the same PCR primers; and

determining whether said ATDC gene contains any mutations by comparing the PCR products of the amplification of said gene with those from the amplification of the normal gene, and detecting differences between the PCR products associated with mutations.

12. A method according to Claim 11 wherein said PCR products are compared for differences in size.

13. A method according to Claim 11 comprising the use of a PCR-single-strand conformation polymorphism assay or a denaturing gradient gel electrophoretic assay to determine whether said ATDC gene contains any mutations.

14. A method according to Claim 11 wherein said PCR primers are selected from the group consisting of the primers shown in Figure 7 [SEQ. ID. NOS.: 4-31] and nucleic acid sequences substantially complementary thereto.

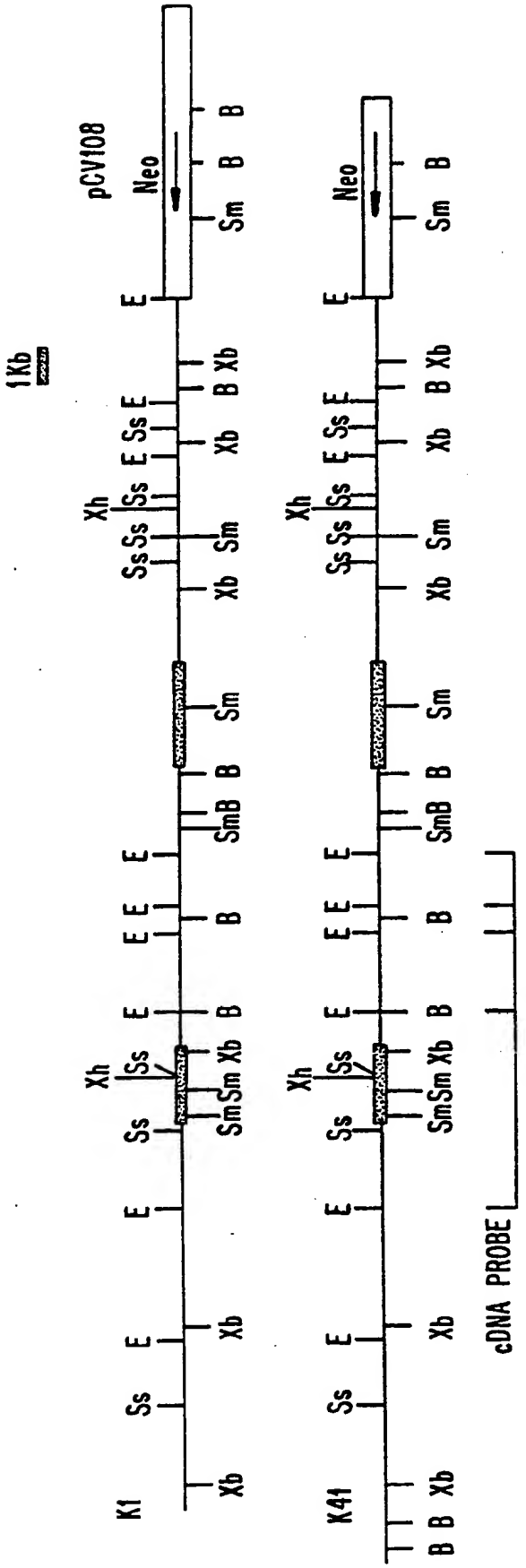
1Kb
pCV108
Neo
E
Sm B B
Xb B Xb
E Ss E
Xb
Xh Ss Ss
Ss Sm
Xb
Sm
SmB B
E E E
B
E E
Xh Ss
Sm Sm Xb B
Ss
E
Xb
K1
Ss
E
Xb
B B Xb
K41
cDNA PROBE

FIG. 1A.

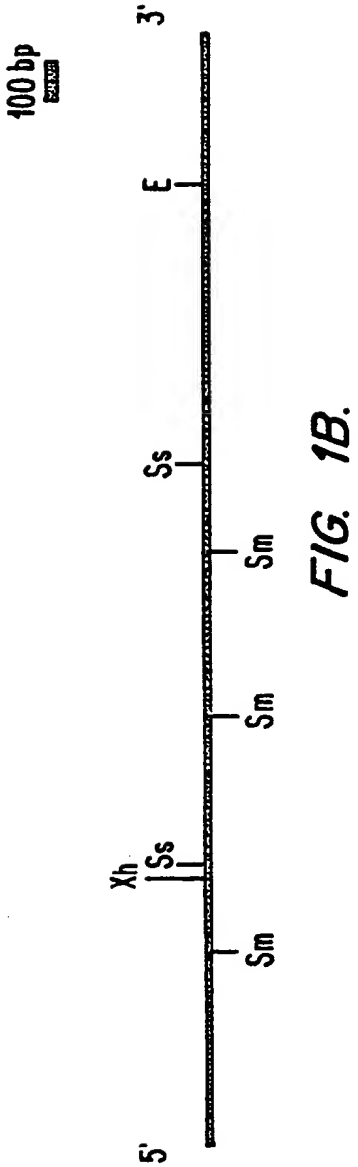
100 bp
3'
E
Ss
Sm
Sm
Xh
Ss
Sm
5'

FIG. 1B.

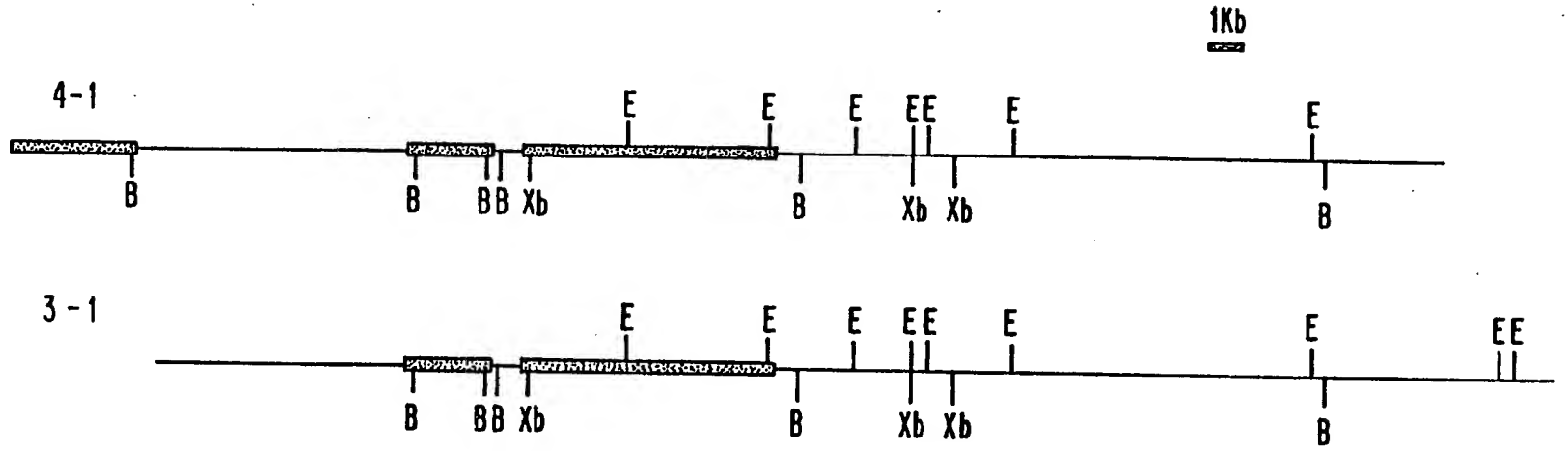

FIG. 2A.

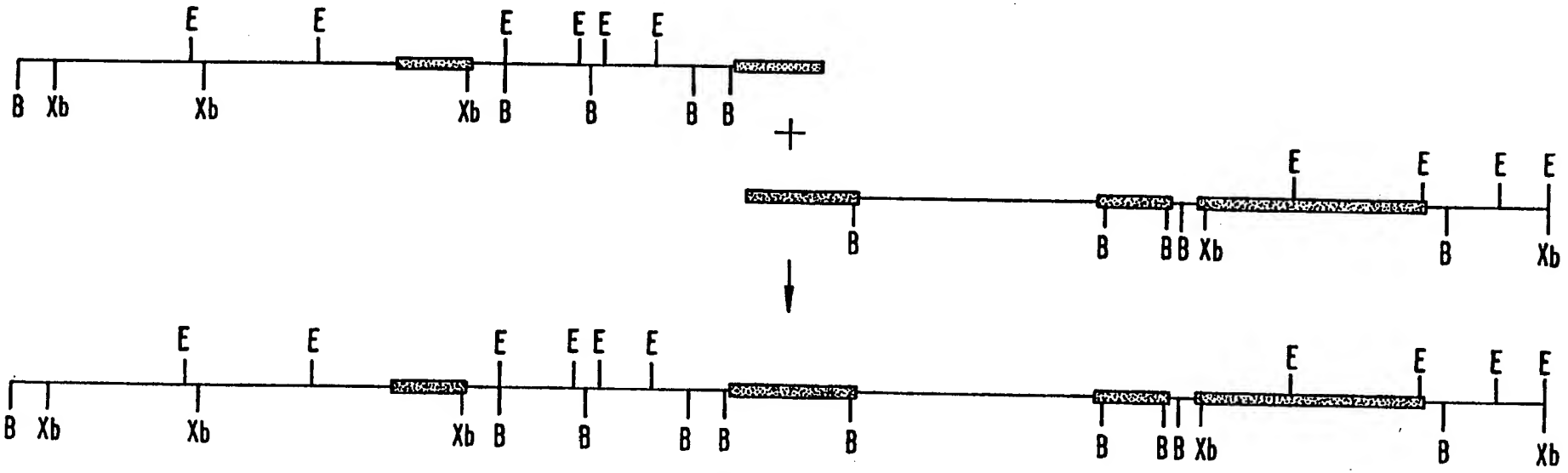

FIG. 2B.

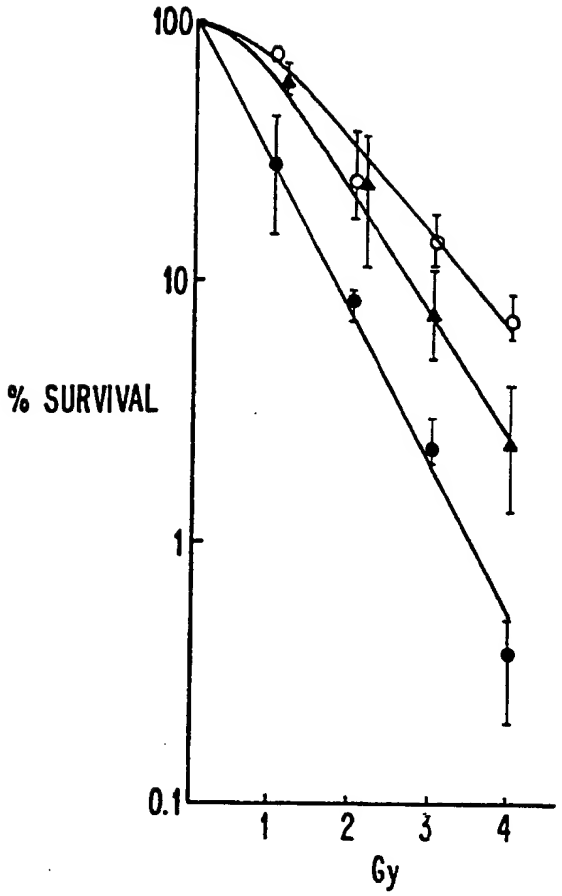

FIG. 3.

```
1     CTCCTCACAG GTGTGTCTCT AGTCCTCGTG GTTGCCTGCC CCACTCCCTG
51    CCGAGACGCC TGCCAGAAAG GTCACCTATC CTGAACCCCA GCAAGCCTGA
                                START          PstI
101   AACAGCTCAG CCAAGCACCC TGCGATGGAA GCTGCAGATG CCTCCAGGAG
151   CAACGGGTCG AGCCCAGAAG CCAGGGATGC CCGGAGCCCG TCGGGCCCCA
201   GTGGCAGCCT GGAGAATGGC ACCAAGGCTG ACGGCAAGGA TGCCAAGACC
251   ACCAACGGGC ACGGCGGGGA GGCAGCTGAG GGCAAGAGCC TGGGCAGCGC
301   CCTGAAGCCA GGGGAAGGTA GGAGCGCCCT GTTCGCGGGC AATGAGTGGC
351   GGCGACCCAT CATCCAGTTT GTCGAGTCCG GGGACGACAA GAACTCCAAC
401   TACTTCAGCA TGGACTCTAT GGAAGGCAAG AGGTCGCCGT ACGCAGGGCT
          PvuI
451   CCAGCTGGGG GCTGCCAAGA AGCCACCCGT TACCTTTGCC GAAAAGGGCG
501   ACGTGCGCAA GTCCATTTTC TCGGAGTCCC GGAAGCCCAC GGTGTCCATC
          SmaI                                  SmaI
551   ATGGAGCCCG GGGAGACCCG GCGGAACAGC TACCCCCGGG CCGACACGGG
601   CCTTTTTTCA CGGTCCAAGT CCGGCTCCGA GGAGGTGCTG TGCGACTCCT
651   GCATCGGCAA CAAGCAGAAG GCGGTCAAGT CCTGCCTGGT GTGCCAGGCC
701   TCCTTCTGCG AGCTGCATCT CAAGCCCCAC CTGGAGGGCG CCGCCTTCCG
                    XhoI
751   AGACCACCAG CTGCTCGAGC CCATCCGGGA CTTTGAGGCC CGCAAGTGTC
                          SstI
801   CCGTGCATGG CAAGACGATG GAGCTCTTCT GCCAGACCGA CCAGACCTGC
851   ATCTGCTACC TTTGCATGTT CCAGGAGCAC AAGAATCATA GCACCGTGAC
                                EXON1|EXON2
901   AGTGGAGGAG GCCAAGGCCG AGAAGGAG|AC GGAGCTGTCA CTGCAAAAGG
          PvuI PstI
951   AGCAGCTGCA GCTCAAGATC ATTGAGATTG AGGATGAAGC TGAGAAGTGG
                                EXON2|EXON3
1001  CAGAAGGAGA AGGACCGCAT CAAG|AGCTTC ACCACCAATG AGAAGGCCAT
1051  CCTGGAGCAG AACTTCCGGG ACCTGGTGCG GGACCTGGAG AAGCAAAAGG
1101  AGGAAGTGAG GGCTGCGCTG GAGCAGCGGG AGCAGGATGC TGTGGACCAA
1151  GTGAAGGTGA TCATGGATGC TCTGGATGAG AGAGCCAAGG TGCTGCATGA
                    SmaI
1201  GGACAAGCAG ACCCGGGAGC AGCTGCATAG CATCAGCGAC TCTGTGTTGT
          EXON3|EXON4
1251  TTCTGCAG|GA ATTTGGTGCA TTGATGAGCA ATTACTCTCT CCCCCCACCC
          PstI
1301  CTGCCCACCT ATCATGTCCT GCTGGAGGGG GAGGGCCTGG GACAGTCACT
```

FIG. 4A.

1351 AGGCAACTTC AAGGACGACC TGCTCAATGT ATGCATGCGC CACGTTGAGA

1401 AGATGTGCAA GGCGGACCTG AGCCGTAACT TCATTGAGAG GAACCACATG

EXON 4 | EXON 5

1451 GAGAACG|GTG GTGACCATCG CTATGTGAAC AACTACACGA ACAGCTTCGG

1501 GGGTGAGTGG AGTGCACCGG ACACCATGAA GAGATACTCC ATGTACCTGA

EXON 5 | EXON 6

1551 CACCCAAAG|G TGGGGTCCGG ACATCATACC AGCCCTCGTC TCCTGGCCGC

1601 TTCACCAAGG AGACCACCCA GAAGAATTTC AACAATCTCT ATGGCACCAA

EXON 6 | EXON 7 SmaI

1651 AG|GTAACTAC ACCTCCCGGG TCTGGGAGTA CTCCTCCAGC ATTCAGAACT

1701 CTGACAATGA CCTGCCCGTC GTCCAAGGCA GCTCCTCCTT CTCCCTGAAA

EXON 7 | EXON 8

1751 G|GCTATCCCT CCCTCATGCG GAGCCAAAGC CCCAAGGCCC AGCCCCAGAC

EXON 8 | EXON 9

1801 TTGGAAATCT GGCAAGCAGA CTATGCTG|TC TCACTACCGG CCATTCTACG

STOP SstI

1851 TCAACAAAGG CAACGGGATT GGGTCCAACG AAGCCCCATG AGCTCCTGGC

1901 GGAAGGAACG AGGCGCCACA CCCCTGCTCT TCCTCCTGAC CCTGCTGCTC

1951 TTGCCTTCTA AGCTACTGTG CTTGTCTGGG TGGGAGGGAG CCTGGTCCTG

PstI

2001 CACCTGCCCT CTGCAGCCCT CTGCCAGCCT CTTGGGGGCA GTTCCGGCCT

2051 CTCCGACTTC CCCACTGGCC ACACTCCATT CAGACTCCTT TCCTGCCTTG

2101 TGACCTCAGA TGGTCACCAT CATTCCTGTG CTCAGAGGCC AACCCATCAC

2151 AGGGGTGAGA TAGGTTGGGG CCTGCCCTAA CCCGCCAGCC TCCTCCTCTC

2201 GGGCTGGATC TGGGGGCTAG CAGTGAGTAC CCGCATGGTA TCAGCCTGCC

2251 TCTCCCGCCC ACGCCCTGCT GTCTCCAGGC CTATAGACGT TTCTCTCCAA

2301 GGCCCTATCC CCCAATGTTG TCAGCAGATG CCTGGACAGC ACAGCCACCC

2351 ATCTCCCATT CACATGGCCC ACCTCCTGCT TCCCAGAGGA CTGGCCCTAC

PstI

2401 GTGCTCTCTC TCGTCCTACC TATCAATGCC CAGCATGGCA GAACCTGCAG

PstI

2451 TGGCCAAGGG CTGCAGATGG AAACCTCTCA GTGTCTTGAC ATCACCCTAC

2501 CCAGGCGGTG GGTCTCCACC ACAGCCACTT TGAGTCTGTG GTCCCTGGAG

2551 GGTGGCTTCT CCTGACTGGC AGGATGACCT TAGCCAAGAT ATTCCTCTGT

EcoRI

2601 TCCCTCTGCT GAGATAAAGA ATTCCCTTAA CATGATATAA TCCACCCATG

2651 CAAATAGCTA CTGGCCCAGC TACCATTTAC CATTTGCCTA CAGAATTTCA

FIG. 4B.

```
2701   TTCAGTCTAC ACTTTGGCAT TCTCTCTGGC GATGGAGTGT GGCTGGGCTG
2751   ACCGCAAAAG GTGCCTTACA CACTGCCCCC ACCCTCAGCC GTTGCCCCAT
2801   CAGAGGCTGC CTCCTCCTTC TGATTACCCC CCATGTTGCA TATCAGGGTG
2851   CTCAAGGATT GGAGAGGAGA CAAAACCAGG AGCAGCACAG TGGGGACATC
2901   TCCCGTCTCA ACAGCCCCAG GCCTATGGGG GCTCTGGAAG GATGGGCCAG
2951   CTTGCAGGGG TTGGGGAGGG AGACATCCAG CTTGGGCTTT CCCCTTTGGA
       POLY A ADDITION SEQUENCE
3001   ATAAACCATT GGTCTGTC - POLY A
```

FIG. 4C.

```
   1                    21                    41
   ATG GAA GCT GCA GAT GCC TCC AGG AGC AAC GGG TCG AGC CCA GAA
1: met glu ala ala asp ala ser arg ser asn gly ser ser pro glu

                    61                    81
   GCC AGG GAT GCC CGG AGC CCG TCG GGC CCC AGT GGC AGC CTG GAG
1: ala arg asp ala arg ser pro ser gly pro ser gly ser leu glu

                101                   121
   AAT GGC ACC AAG GCT GAC GGC AAG GAT GCC AAG ACC ACC AAC GGG
1: asn gly thr lys ala asp gly lys asp ala lys thr thr asn gly

          141                   161
   CAC GGC GGG GAG GCA GCT GAG GGC AAG AGC CTG GGC AGC GCC CTG
1: his gly gly glu ala ala glu gly lys ser leu gly ser ala leu

   181                   201                   221
   AAG CCA GGG GAA GGT AGG AGC GCC CTG TTC GCG GGC AAT GAG TGG
1: lys pro gly glu gly arg ser ala leu phe ala gly asn glu trp

                    241                   261
   CGG CGA CCC ATC ATC CAG TTT GTC GAG TCC GGG GAC GAC AAG AAC
1: arg arg pro ile ile gln phe val glu ser gly asp asp lys asn

                281                   301
   TCC AAC TAC TTC AGC ATG GAC TCT ATG GAA GGC AAG AGG TCG CCG
1: ser asn tyr phe ser met asp ser met glu gly lys arg ser pro

          321                   341
   TAC GCA GGG CTC CAG CTG GGG GCT GCC AAG AAG CCA CCC GTT ACC
1: tyr ala gly leu gln leu gly ala ala lys lys pro pro val thr

   361                   381                   401
   TTT GCC GAA AAG GGC GAC GTG CGC AAG TCC ATT TTC TCG GAG TCC
1: phe ala glu lys gly asp val arg lys ser ile phe ser glu ser

                    421                   441
   CGG AAG CCC ACG GTG TCC ATC ATG GAG CCC GGG GAG ACC CGG CGG
1: arg lys pro thr val ser ile met glu pro gly glu thr arg arg
```

FIG. 5A.

```
                461                             481
   AAC AGC TAC CCC CGG GCC GAC ACG GGC CTT TTT TCA CGG TCC AAG
1: asn ser tyr pro arg ala asp thr gly leu phe ser arg ser lys

        501                             521
   TCC GGC TCC GAG GAG GTG CTG TGC GAC TCC TGC ATC GGC AAC AAG
1: ser gly ser glu glu val leu cys asp ser cys ile gly asn lys

   541                             561                             581
   CAG AAG GCG GTC AAG TCC TGC CTG GTG TGC CAG GCC TCC TTC TGC
1: gln lys ala val lys ser cys leu val cys gln ala ser phe cys

                        601                             621
   GAG CTG CAT CTC AAG CCC CAC CTG GAG GGC GCC GCC TTC CGA GAC
1: glu leu his leu lys pro his leu glu gly ala ala phe arg asp

                641                             661
   CAC CAG CTG CTC GAG CCC ATC CGG GAC TTT GAG GCC CGC AAG TGT
1: his gln leu leu glu pro ile arg asp phe glu ala arg lys cys

        681                             701
   CCC GTG CAT GGC AAG ACG ATG GAG CTC TTC TGC CAG ACC GAC CAG
1: pro val his gly lys thr met glu leu phe cys gln thr asp gln

   721                             741                             761
   ACC TGC ATC TGC TAC CTT TGC ATG TTC CAG GAG CAC AAG AAT CAT
1: thr cys ile cys tyr leu cys met phe gln glu his lys asn his

                        781                             801
   AGC ACC GTG ACA GTG GAG GAG GCC AAG GCC GAG AAG GAG ACG GAG
1: ser thr val thr val glu glu ala lys ala glu lys glu thr glu

                821                             841
   CTG TCA CTG CAA AAG GAG CAG CTG CAG CTC AAG ATC ATT GAG ATT
1: leu ser leu gln lys glu gln leu gln leu lys ile ile glu ile

        861                             881
   GAG GAT GAA GCT GAG AAG TGG CAG AAG GAG AAG GAC CGC ATC AAG
1: glu asp glu ala glu lys trp gln lys glu lys asp arg ile lys
```

FIG. 5B.

901 921 941
AGC TTC ACC ACC AAT GAG AAG GCC ATC CTG GAG CAG AAC TTC CGG
1: ser phe thr thr asn glu lys ala ile leu glu gln asn phe arg

961 981
GAC CTG GTG CGG GAC CTG GAG AAG CAA AAG GAG GAA GTG AGG GCT
1: asp leu val arg asp leu glu lys gln lys glu glu val arg ala

1001 1021
GCG CTG GAG CAG CGG GAG CAG GAT GCT GTG GAC CAA GTG AAG GTG
1: ala leu glu gln arg glu gln asp ala val asp gln val lys val

1041 1061
ATC ATG GAT GCT CTG GAT GAG AGA GCC AAG GTG CTG CAT GAG GAC
1: ile met asp ala leu asp glu arg ala lys val leu his glu asp

1081 1101 1121
AAG CAG ACC CGG GAG CAG CTG CAT AGC ATC AGC GAC TCT GTG TTG
1: lys gln thr arg glu gln leu his ser ile ser asp ser val leu

1141 1161
TTT CTG CAG GAA TTT GGT GCA TTG ATG AGC AAT TAC TCT CTC CCC
1: phe leu gln glu phe gly ala leu met ser asn tyr ser leu pro

1181 1201
CCA CCC CTG CCC ACC TAT CAT GTC CTG CTG GAG GGG GAG GGC CTG
1: pro pro leu pro thr tyr his val leu leu glu gly glu gly leu

1221 1241
GGA CAG TCA CTA GGC AAC TTC AAG GAC GAC CTG CTC AAT GTA TGC
1: gly gln ser leu gly asn phe lys asp asp leu leu asn val cys

1261 1281 1301
ATG CGC CAC GTT GAG AAG ATG TGC AAG GCG GAC CTG AGC CGT AAC
1: met arg his val glu lys met cys lys ala asp leu ser arg asn

1321 1341
TTC ATT GAG AGG AAC CAC ATG GAG AAC GGT GGT GAC CAT CGC TAT
1: phe ile glu arg asn his met glu asn gly gly asp his arg tyr

FIG. 5C.

```
                1361                                1381
   GTG AAC AAC TAC ACG AAC AGC TTC GGG GGT GAG TGG AGT GCA CCG
1:  val asn asn tyr thr asn ser phe gly gly glu trp ser ala pro

        1401                                1421
   GAC ACC ATG AAG AGA TAC TCC ATG TAC CTG ACA CCC AAA GGT GGG
1:  asp thr met lys arg tyr ser met tyr leu thr pro lys gly gly

   1441                        1461                        1481
   GTC CGG ACA TCA TAC CAG CCC TCG TCT CCT GGC CGC TTC ACC AAG
1:  val arg thr ser tyr gln pro ser ser pro gly arg phe thr lys

                        1501                        1521
   GAG ACC ACC CAG AAG AAT TTC AAC AAT CTC TAT GGC ACC AAA GGT
1:  glu thr thr gln lys asn phe asn asn leu tyr gly thr lys gly

                1541                                1561
   AAC TAC ACC TCC CGG GTC TGG GAG TAC TCC TCC AGC ATT CAG AAC
1:  asn tyr thr ser arg val trp glu tyr ser ser ser ile gln asn

        1581                                1601
   TCT GAC AAT GAC CTG CCC GTC GTC CAA GGC AGC TCC TCC TTC TCC
1:  ser asp asn asp leu pro val val gln gly ser ser ser phe ser

   1621                        1641                        1661
   CTG AAA GGC TAT CCC TCC CTC ATG CGG AGC CAA AGC CCC AAG GCC
1:  leu lys gly tyr pro ser leu met arg ser gln ser pro lys ala

                        1681                        1701
   CAG CCC CAG ACT TGG AAA TCT GGC AAG CAG ACT ATG CTG TCT CAC
1:  gln pro gln thr trp lys ser gly lys gln thr met leu ser his

                1721                                1741
   TAC CGG CCA TTC TAC GTC AAC AAA GGC AAC GGG ATT GGG TCC AAC
1:  tyr arg pro phe tyr val asn lys gly asn gly ile gly ser asn

        1761
   GAA GCC CCA TGA
1:  glu ala pro ***
```

FIG. 5D.

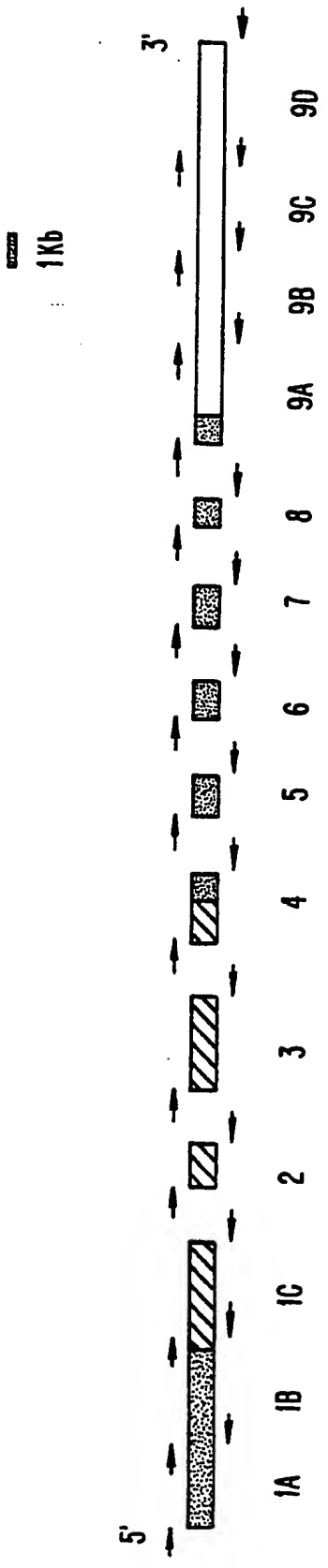

FIG. 6.

FRAGMENT	PRIMER	SEQUENCE
1A	1A-5	TCTCTAGTCCTCGTGGTT
	1A-3	GGTGGTCTTGGCATCCTT
1B	1B-5	TGGAGAATGGCACCAAGG
	1B-3	TCCATGATGGACACCGTG
1C	1C-5	TCTATGGAAGGCAAGAGG
	1C-3	GGAGAAGATGAAGTTCGG
2	2-5	TGACTTCTCCAATCCTGG
	2-3	CCTGGACTCAAATGGGAG
3	3-5	AAGACATACCCGACTAGG
	3-3	TGTGAAATCGAGGGCTTG
4	4-5	AGCGTCCTCATAGCTCAT
	4-3	TGAGAAGAAGCTCACTGG
5	5-5	AAACTTGGATCTGCCTGG
	5-3	AGTCACTGCACGGACTTT
6	6-5	GAGTCCTGATGAGACAAT
	6-3	CATTCATCTCACACTGGG
7	7-5	AGAGAGTCATAGACCTGG
	7-3	GAGGAACTAGCAGCTCAG
8	8-5	GACGGCTGCATTTGGTAA
	8-3	CAGAGAAGTCCTCCCACA
9A	9A-5	AGAATTGTCGGGTCTTGG
	9A-3	GCACAGTAGCTTAGAAGG
9B	9B-5	ACAAAGGCAACGGGATTG
	9B-3	TCTGCTGACAACATTGGG
9C	9C-5	AGACGTTTCTCTCCAAGG
	9C-3	CTTTATCTCAGCAGAGGG
9D	9D-5	AGGATGACCTTAGCCAAG
	9D-3	GAAGAACTGCAGCCTGTT

FIG. 7.

1A
(SEQUENCED STRAND)

IA-5

1 TCTCTAGTCCTCGTGGTTGCCTGCCCCACTCCCTGCCGAG

41 ACGCCTGCCAGAAAGGTCACCTATCCTGAACCCCAGCAAG

START PstI

81 CCTGAAACAGCTCAGCCAAGCACCCTGCGATGGAAGCTGC

121 AGATGCCTCCAGGAGCAACGGGTCGAGCCCAGAAGCCAGG

161 GATGCCCGGAGCCCGTCGGGCCCCAGTGGCAGCCTGGAGA

201 ATGGCACCAAGGCTGACGGCAAGGATGCCAAGACCACC

IA-3

FIG. 8A.

1B
(SEQUENCED STRAND)

IB-5

1 TGGAGAATGGCACCAAGGCTGACGGCAAGGATGCCAAGAC

41 CACCAACGGGCACGGCGGGGAGGCAGCTGAGGGCAAGAGC

81 CTGGGCAGCGCCCTGAAGCCAGGGGAAGGTAGGAGCGCCC

121 TGTTCGCGGGCAATGAGTGGCGGCGACCCATCATCCAGTT

161 TGTCGAGTCCGGGGACGACAAGAACTCCAACTACTTCAGC

201 ATGGACTCTATGGAAGGCAAGAGGTCGCCGTACGCAGGGC

PvuI

241 TCCAGCTGGGGGCTGCCAAGAAGCCACCCGTTACCTTTGC

281 CGAAAAGGGCGACGTGCGCAAGTCCATTTTCTCGGAGTCC

321 CGGAAGCCCACGGTGTCCATCATGGA

IB-3

FIG. 8B.

1C
(SEQUENCED STRAND)

1C-5

1 TCTATGGAAGGCAAGAGGTCGCCGTACGCAGGGCTCCAGC

41 TGGGGGCTGCCAAGAAGCCACCCGTTACCTTTGCCGAAAA

81 GGGCGACGTGCGCAAGTCCATTTTCTCGGAGTCCCGGAAG

Sma I
121 CCCACGGTGTCCATCATGGAGCCCGGGGAGACCCGGCGGA

Sma I
161 ACAGCTACCCCGGGCCGACACGGGCCTTTTTTCACGGTC

201 CAAGTCCGGCTCCGAGGAGGTGCTGTGCGACTCCTGCATC

241 GGCAACAAGCAGAAGGCGGTCAAGTCCTGCCTGGTGTGCC

281 AGGCCTCCTTCTGCGAGCTGCATCTCAAGCCCCACCTGGA

Xho I
321 GGGCGCCGCCTTCCGAGACCACCAGCTGCTCGAGCCCATC

361 CGGGACTTTGAGGCCCGCAAGTGTCCCGTGCATGGCAAGA

Sst I
401 CGATGGAGCTCTTCTGCCAGACCGACCAGACCTGCATCTG

441 CTACCTTTGCATGTTCCAGGAGCACAAGAATCATAGCACC

EXON 1 | INTRON
481 GTGACAGTGGAGGAGGCCAAGGCCGAGAAGGAG|GTAAGTG

521 CTGGGGCCCCTCCTGCCCCTCCAGGCCTCTCCTCTCTCAA

561 CCCACCCCTCCGAACTTCATCTTCTCC

1C-3

FIG. 8C.

2
(SEQUENCED STRAND)

2-5 →

INTRON | EXON2

1 TGACTTCTCCAATCCTGGCTCTTTCTCTGCAG|ACGGAGCT

Pst I Pst I

41 GTCACTGCAAAAGGAGCAGCTGCAGCTCAAGATCATTGAG

81 ATTGAGGATGAAGCTGAGAAGTGGCAGAAGGAGAAGGACC

EXON 2 | INTRON

121 GCATCAAG|GTGAGCAGCCCCCAAGCTCACCTTGCTGCTCC

161 CTTACCCGACCTGGCCTGCCTGGAAAGACGCAGGCCTTGG

201 CTCCCATTTGAGTCCAGG

2-3

FIG. 8D.

3
(SEQUENCED STRAND)

3-5 →

1 AAGACATACCCGACTAGGGTGATTTCTTTCCCTAACTAAA

INTRON | EXON3

41 GCCCTGCCTAATCTCTTCCCTGACTCTGGACCTCCAG|AGC

81 TTCACCACCAATGAGAAGGCCATCCTGGAGCAGAACTTCC

121 GGGACCTGGTGCGGGACCTGGAGAAGCAAAAGGAGGAAGT

161 GAGGGCTGCGCTGGAGCAGCGGGAGCAGGATGCTGTGGAC

201 CAAGTGAAGGTGATCATGGATGCTCTGGATGAGAGAGCCA

241 AGGTGCTGCATGAGGACAAGCAGACCCGGGAGCAGCTGCA

EXON3 | INTRON

281 TAGCATCAGCGACTCTGTGTTGTTTCTGCAG|GTAACAAGC

321 CACTCCTCTGTCACTCAAGCCCTCGATTTCACA

3-3

FIG. 8E.

4
(SEQUENCED STRAND)

4-5

```
1    AGCGTCCTCATAGCTCATGAAGACCCAGGCAGTTAATGGT
                INTRON | EXON 4
41   TCTTTCCTTTCTTGGTAG|GAATTTGGTGCATTGATGAGCA
81   ATTACTCTCTCCCCCCACCCCTGCCCACCTATCATGTCCT
121  GCTGGAGGGGGAGGGCCTGGGACAGTCACTAGGCAACTTC
161  AAGGACGACCTGCTCAATGTATGCATGCGNCACGTTGAGA
201  AGATGTGCAAGGCGNACCTGAGCNGTAACTTCATTGAGAG
                EXON 4 | INTRON
241  GAACCACATGGAGAACG|GTAGGTCCCCTCTCGTGGCTGGG
281  CCCCAAGGCNATAGACCTTTCTCTCCCAAATCAATTCCTG
321  CTGCCTGACATGGGCTGGCCTCCAGTGAGCTTCTTCTCA
```

4-3

FIG. 8F.

5
(SEQUENCED STRAND)

5-5

```
1    AAACTTGGATCTGCCTGGGAGATAGGGGAAGGGCTATGGG
41   GTGACTCATCTGAGCCCCAAAAGTCCCCAGTGGCTGGCTC
81   CTCCTTCCCACCTGGCTCCTCTGCTGACCCGACCCTCTGC
          INTRON | EXON 5
121  TTCCTAG|GTGGTGACCATCGCTATGTGAACAACTACACGA
161  ACAGCTTCGGGGGTGAGTGGAGTGCACCGGACACCATGAA
                          EXON5 | INTRON
201  GAGATACTCCATGTACCTGACACCCAAAG|GTAAGAGGGAG
241  CCCCTCACCCCAGACCTAGTGTCTCTCCTGCTGCCCAGGG
281  GCCCCCAAAGTCCGTGCAGTGACT
```

5-3

FIG. 8G.

6
(SEQUENCED STRAND)

6-5

1 GAGTCCTGATGAGACAATTTTGTGCAATGACAGCCCNNTT

INTRON | EXON6

41 CATCTGCTTCACAG|GTGGGGTCCGGACATCATACCAGCCC

81 TCGTCTCCTGGCCGCTTCACCAAGGAGACCACCCAGAAGA

EXON6 | INTRON

121 ATTTCAACAATCTCTATGGCACCAAAG|NNNNNNNTGGGNC

161 TGTGCAGGCAGGAGGGCATAGAGGTGGGTCCAGNGGCACA

201 GGGCTGGGACCCCAGTGTGAGATGAATG

6-3

FIG. 8H.

7
(SEQUENCED STRAND)

7-5

1 AGAGAGTCATAGACCTGGCTGTGTCCTGGTCCTGCCTCCT

41 CTCCCACTCCCAGCTGTGGGGGCCTGACAGCCCTTCTTTG

INTRON | EXON7 SmaI

81 TCCTGCCAG|GTAACTACACCTCCCGGGTCTGGGAGTACTC

121 CTCCAGCATTCAGAACTCTGACAATGACCTGCTGTCGTCC

EXON 7 | INTRON

161 AAGGCAGCTCCTCCTTCTCCCTGAAAG|GTGAGCCCTGCCC

201 ACCCTGGCCCCTGCTTTCCTCCACAGCTGCCTCACACCTC

241 CCAAGCCCTGCTTGGGTCTCTTCGCTGAGCTGCTAGTTCC

7-3

281 TC

FIG. 8I.

8
(SEQUENCED STRAND)

8-5

1 GACGGCTGCATTTGGTAATGGGCTGGATGATGCTTGGTGG

41 TACACTTTGGAGAAGNAGCTGTGCTGCTCTGGGNCCGGGN

81 NCCCCTGGCCNNNNNNNNNNNNNNNNNNNNNNNNNNNN

INTRON | EXON 8

121 NNNNNNNNNNNN|GCTATCCCTCCCTCATGCGGAGCCAAAG

161 CCCCAAGGCCCAGCCCCAGACTTGGAAATCTGGCAAGCAG

EXON 8 | INTRON

201 ACTATGCTG|GTAAGGGAAGTGCGGCCGGGAGGGCCTGGGC

241 ACATCCAGAGACCTGGGCACTGAAGGGGGCTCCCTGGAGG

281 CAATCGGTTCCAGGGCCTGTGGGAGGACTTCTCTG

8-3

FIG. 8J.

9A
(UNSEQUENCED STRAND)

9A-5

1 AGAATTGTCGGGTCTTGGATCACTGCTGCCTCCTGAGGCA

41 GGTTAGGGTAGGGTGGGTCTAGCTAGCAGGCTCATCTGTC

INTRON | EXON 9

81 GTCTGGCCTCGCTGACCACTCTTGTTTCCCCCACAG|TCTC

121 ACTACCGGCCATTCTACGTCAACAAAGGCAACGGGATTGG

STOP SstI

161 GTCCAACGAAGCCCCA[TGA]GCTCCTGGCGGAAGGAACGAG

201 GCGCCACACCCCTGCTCTTCCTCCTGACCCTGCTGCTCTT

241 GCCTTCTAAGCTACTGTGC

9A-3

FIG. 8K.

9B
(SEQUENCED STRAND)

9B-5

```
1    ACAAAGGCAACGGGATTGGGTCCAACGAAGCCCCATGAGC
41   TCCTGGCGGAAGGAACGAGGCGCCACACCCCTGCTCTTCC
81   TCCTGACCCTGCTGCTCTTGCCTTCTAAGCTACTGTGCTT
121  GYCTGGGTGGGAGGGAGCCTGGTCCTGCACCTGCCCTCTG
                                        PstI
161  CAGCCCTCTGCCAGCCTCTTGGGGGCAGTTCCGGCCTCTC
201  CGACTTCCCCACTGGCCACACTCCATTCAGACTCCTTTCC
241  TGCCTTGTGACCTCAGATGGTCACCATCATTCCTGTGCTC
281  AGAGGCCAACCCATCACAGGGGTGAGATAGGTTGGGGCCT
321  GCCCTAACCCGCCAGCCTCCTCCTCTCGGGCTGGATCTGG
361  GGGCTAGCAGTGAGTACCCGCATGGTATCAGCCTGCCTCT
401  CCCGCCCACGCCCTGCTGTCTCCAGGCCTATAGACGTTTC
441  TCTCCAAGGCCCTATCCCCCAATGTTGTCAGCAGA
```

9B-3

FIG. 8L.

9C
(SEQUENCED STRAND)

9C-5

1 AGACGTTTCTCTCCAAGGCCCTATCCCCCAATGTTGTCAG

41 CAGATGCCTGGACAGCACAGCCACCCATCTCCCATTCACA

81 TGGCCCACCTCCTGCTTCCCAGAGGACTGGCCCTACGTGC

121 TCTCTCTCGTCCTACCTATCAATGCCCAGCATGGCAGAAC

PstI PstI
161 CTGCAGTGGCCAAGGGCTGCAGATGGAAACCTCTCAGTGT

201 CTTGACATCACCCTACCCAGGCGGTGGGTCTCCACCACAG

241 CCACTTTGAGTCTGTGGTCCCTGGAGGGTGGCTTCTCCTG

281 ACTGGCAGGATGACCTTAGCCAAGATATTCCTCTGTTCCC

321 TCTGCTGAGATAAAG

9C-3

FIG. 8M.

9D
(SEQUENCED STRAND)

9D-5

1 AGGATGACCTTAGCCAAGATATTCCTCTGTTCCCTCTGCT

EcoRI

41 GAGATAAAGAATTCCCTTAACATGATATAATCCACCCATG

81 CAAATAGCTACTGGCCCAGCTACCATTTACCATTTGCCTA

121 CAGAATTTCATTCAGTCTACACTTTGGCATTCTCTCTGGC

151 GATGGAGTGTGGCTGGGCTGACCGCAAAAGGTGCCTTACA

201 CACTGCCCCCACCCTCAGCCGTTGCCCCATCAGAGGCTGC

241 CTCCTCCTTCTGATTACCCCCCATGTTGCATATCAGGGTG

281 CTCAAGGATTGGAGAGGAGACAAAACCAGGAGCAGCACAG

321 TGGGGACATCTCCCGTCTCAACAGCCCCAGGCCTATGGGG

361 GCTCTGGAAGGATGGGCCAGCTTGCAGGGGTTGGGGAGGG

401 AGACATCCAGCTTGGGCTTTCCCCTTTGGAATAAACCATT

cDNA - END

441 GGTCTGTCACTTCTCTTGTATTGAATGACCATTTCCCTGA

481 GGGTCCCCAGAGGAACAGGCTGCAGTTCTTC

9D-3

FIG. 8N.